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MISCHA E. FRIEDMAN
U.S. Army Biological Center, Fort Detrick, Frederick, Maryland

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Enterotoxin B synthesis by *Staphylococcus aureus* S6 in broth cultures was inhibited by the addition to the medium of culture filtrates from several media, particularly Brain Heart Infusion (E. Mullett, Jr., and M. E. Friedman, Bacteriol. Proc., p. 108, 1961). It was shown later that the responsible factor was not a growth product and that a variety of known compounds could inhibit enterotoxin synthesis (Table 1). The medium, 25 ml per 250-ml Erlenmeyer flask, consisted of 2% Protein Hydrolysate Powder (Mead Johnson & Co., Evansville, Ind.), 0.001% thiamine, and 0.001% nicotinic acid, and was adjusted initially to *p*H 7.7. The inhibitors, except for streptomycin and chloramphenicol which were added aseptically to prepared media, were added with the other medium constituents. Incubation was at 37 C with shaking for 16 hr. Enterotoxin in pooled culture filtrates obtained by ultrafine filtration through glass was assayed by the single agar-diffusion technique (J. Oudin, Compt. Rend. **222**:115, 1946; S. J. Silverman, J. Bacteriol. **85**: 955, 1963).

The activity of the inorganic salts and acriflavine suggested a relationship of lysogeny to toxinogenicity. However, the work of R. B. Read, Jr., and W. L. Pritchard (Bacteriol. Proc., p. 15, 1963) with cured cells which retained toxinogenicity, and our own studies with nonproducing cells which did not synthesize toxin after lysogenization with bacteriophage isolated from strain S6, strongly indicated that such a relationship did not exist.

Inhibition was diminished progressively if the *p*H of the medium was adjusted initially to 7.3 or lower in the presence of K_2HPO_4 or to 7.5 or lower in the presence of KCl. Complete inhibition did occur, however, at *p*H 7.0 if the concentration of KCl was increased 25-fold over that required in a medium at *p*H 7.7. Acriflavine and spermine activities were affected similarly. Other antagonists of some but not all of the inhibitors were NH_4^+, Mg^{++}, and Ca^{++} salts (Table 2). The relationship between K^+ and NH_4^+ was competitive.

If K^+ ions were added to the culture 3 or 4 hr after inoculation, the inhibitory concentration was increased threefold over the zero-time requirement, even though no measurable toxin was found in the medium for an additional 2 to 3 hr. Subculture of strain S6 in the presence of K^+, acriflavine, or phenethyl alcohol failed to cause a loss in enterotoxin-synthesizing ability, because subsequent subculture without the inhibitors resulted in the usual toxin yield. Sonic disruption or lysis by shaking at 4 C for 24 hr of either young or old cells did not reveal any significant amount of intracellular enterotoxin, and cells grown in

TABLE 1. *Inhibition of staphylococcal enterotoxin B synthesis*[a]

Inhibitor	Amt (μmoles/ml)	OD[b] (655 mμ)	Enterotoxin (μg/ml)	Inhibition (%)
K_2HPO_4	0	.35	45	
	36.74	.36	0	100
KCl	53.66	.35	0	100
$CoCl_2$	0	.33	35	
	1.68	.30	0	100
NaF	0	.37	73	
	57.14	.21	0	100
Acriflavine	0	.28	63	
	0.002[c]	.34	0	100
Phenethyl alcohol	0	.32	39	
	16.37	.39	8	79
Streptomycin sulfate	0	.35	45	
	0.0007	.35	0	100
Chloramphenicol	0	.26	61	
	0.009	.22	26	57
Spermine phosphate	0	.39	50	
	0.30	.34	0	100
Spermidine phosphate	0	.33	31	
	0.18	.35	0	100
Tween 80	0	.33	39	
	6.0[c]	.44	0	100

[a] After 16 hr of incubation.
[b] Optical density of 10-fold diluted culture.
[c] Milligrams per milliliter.

TABLE 2. *Inhibitors of staphylococcal enterotoxin B synthesis and reversal*[a]

Inhibitor	Inhibition reversed by			
	NH_4^+	Mg^{++}	Ca^{++}	Reduced initial pH
K_2HPO_4	+	±	NT	+
KCl	+	±	+	+
$CoCl_2$	+	±	±	±
NaF	+	+	−	−
Acriflavine	+	+	+	+
Phenethyl alcohol	+	−	−	−
Streptomycin sulfate	+	+	+	−
Chloramphenicol	−	−	−	−
Spermine phosphate	+	+	+	+
Tween 80	−	+	+	±

[a] Symbols: + = >50% reversal; ± = <50% reversal; − = inactive; NT = not tested.

the presence of inhibitory concentrations of K^+, spermine, or Tween 80 did not accumulate toxin. Further, cells grown in the presence of Tween 80 exhibited no fluorescence when stained by the immunofluorescence technique described by M. E. Friedman and J. D. White (J. Bacteriol. **89**: 1155, 1965).

The mechanism of inhibition is not known; however, it is probable that acriflavine (J. D. Leith, Jr., Biochim. Biophys. Acta **72**:643, 1963) and phenethyl alcohol (G. Berrah and W. A. Konetzka, J. Bacteriol. **83**:738, 1962) affect deoxyribonucleic acid synthesis. The inhibitions by K^+ and NaF and reversal of each by Mg^{++} suggest that the enzymatic system controlling enterotoxin B synthesis may require Mg^{++}. NaF inhibits enzymatic reactions by complexing with Mg^{++}, and the uptake of Mg^{++} in plants was inhibited by K^+ (K. Scharrer and K. Mengel, Z. Pflanzenernaehr. Dueng. Bodenk. **83**:149, 1960). Co^{++} may act with serine to retard *S. aureus* metabolism (E. D. Weinberg, Antonie van Leewenhoek J. Microbiol. Serol. **26**:321, 1960). Chloramphenicol (E. F. Gale and J. P. Folkes, Biochem. J. **53**:493, 1953) and streptomycin (T. Erdos and A. Ullmann, Nature **183**:618, 1959) inhibit protein synthesis, and S. M. Friedman and J. B. Weinstein (Proc. Natl. Acad. Sci. U. S. **52**:988, 1964) demonstrated that spermine may act in a similar manner as streptomycin. These compounds as well as Tween 80 may affect the permeability of the cells and thus the synthesis of cellular products. Further, the activity of most of the inhibitors may be selective toward enterotoxin since they exhibit little or no activity toward α-lysin or coagulase.